

Silver Thin Films for Ascorbic Acid Sensing

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Abstract

Ascorbic Acid (Vitamin C) is present in a large variety of commercial fruit juices. Their presence can cause changes in the chemical characteristics of the food, such as pH, total acidity and their levels change with storage. Hence, there is a need for having an adequate control and quantification of ascorbic acid to verify the authenticity and quality of the fruits used in food manufacturing. In our project, we suggest a thin film resistor based sensing of Ascorbic acid for such purposes. Copper Hexacyanoferrate (III) ($\text{Cu}_3[\text{Fe}(\text{CN})_6]_2$), a Prussian blue analogue is used as the sensing element. A thin film resistor is fabricated by thermally evaporating silver onto a cleaned glass substrate. The sensing element is then spin coated on the thin film. The dimensions of the resistor being $2\text{cm} \times 1.5\text{cm} \times 1.5\mu\text{m}$ with 1 cm gap between the leads kept by silver paste. The resistor was tested for the change in resistance with respect to different concentrations of Ascorbic acid in open circuit and closed circuit conditions using an electrometer. The working range of the sensor was found to be 0-0.5mg/ml and resistance drop from $\text{G}\Omega$ to $\text{M}\Omega$ was observed when biased with 50 mV. The drop in the resistance was correlated with spectrophotometric results. Reduction in absorbance with different concentrations of Ascorbic acid was observed at 473nm. The basis of detection is mainly the oxidation of Ascorbic acid to Dehydroascorbate in the presence of Copper Hexacyanoferrate, with the latter getting reduced to Cuprous Hexacyanoferrate (II).

Keywords

Sensor; Ascorbic Acid; Copper Hexacyanoferrate; Thermal Evaporation; Spin Coating

Introduction

Thin films are layers of any material whose dimensions vary from nanometres to several micrometers. Thin films are primarily used for optical coatings and semiconductor electronic appliances. Nowadays, thin films are increasingly used for sensor applications. Thin films have been used as transducers for gas sensors, (Ganesh Kumar Mani and John Bosco, 2013, Sudeep Joshi et al. 2013), pressure sensors (C. Lopes, C. Gonçalves et al., 2013, Antonin Ollagnier et al., 2013) and chemical sensors (Engin Çiftiyürek et al., 2013). Metal thin films have been proven to be effective in

conductometric sensing of urea, glucose with very high sensitivity (Shul'ga et al., 1991, G. Urban et al., 1994).

Ascorbic acid (AA) is one of most important biological compounds for human metabolism. The major physiological functions includes the hydroxylation of proline and lysine. Vitamin-C, AA, is the cofactor for the enzyme lysyl oxidase, lysyl hydroxylase and prolyl hydroxylase (J C Murray et al., 1977). AA, being an anti-oxidant, is used to treat some illnesses such as the common cold, cancer and AIDS (Francis FJ, 1999). The antioxidant property of Vitamin C is also used in the food and beverage industry. Oxidation of AA takes place readily on exposure to air thus leading to the degradation of it (Fornaro A and Coichev N, 1998). Time of storage and temperature are important factors in retention of AA as with time and high temperature, the oxidation of Vitamin-C is more (M. S. Uddin et al., 2002 andrea Stešková et al, 2006). Consequently, the accurate determination of its concentration is of considerable importance (J. C. Bauernfeind and D. M. Pinkert 1970).

Many analytical methods for AA determination are already described. They mainly include chromatographic, spectrophotometric, titrimetric, enzymatic and electrochemical methods (M. Arvand et al., 2003). The electrochemical methods proposed are based on modifying electrodes for sensing AA (Suw Young Ly et al., 2004, Jahan-Bakhsh Raoof et al., 2007, Barsan MM, Brett CM, 2009).

Although AA is a readily oxidisable compound, some compounds, particularly hexacyanoferrates, catalyze the oxidation of AA. Hexacyanoferrate (III) reacts with AA chemically in acidic medium (Martins LJA and da Costa JB, 1988) and this reaction is used to determine AA spectrophotometrically in pharmaceutical products (Nu" brega JA and Lopes JS, 1996, Koncki R and Wolfbeis OS, 1998, Koncki R, Lenarczuk T 1999). Prussian blue films have also been found to have an electrocatalytic effect on the oxidation of AA (Li FB and Dong SJ, 1987, Totir N et al., 2001, Hart JP et al., 2004) and they have also been used for its

determination (De Mattos IL and Gorton L 2001). An amperometric flow-injection sensor for AA was developed using a Prussian blue film on a glassy carbon electrode, such a sensor was stable, sensitive and selective in acidic media (Castro SSL et al., 2001). Since Prussian blue films were not sufficiently stable in neutral media, they are not suitable for ascorbate determination at pH 7. It was demonstrated in (Shankaran DR and Narayanan SS, 1999) that AA is easily oxidized at cupric hexacyanoferrate and also used to determine levels of ascorbic acid amperometrically by chemical deposition on carbon electrodes (Rasa Pauliukaite Æ et al., 2003). Ferricyanide had also been used as a mediator in carbonate microsphere electrochemical sensing of AA (Feng Li et al., 2010).

Our goal was to construct a simple thin film sensor for AA sensing in fruit juices and beverages. The amperometric methods already available have their advantages in the high sensitivity and low cost, but involves complex steps to modify and characterize the electrodes. A new idea of using thin films for a much simpler way of sensing ascorbic acid with retention of the advantages of amperometric sensing. The advantages of this new sensor for AA are low cost, low sample volume, simple sensing scheme and sensitivity of the sensor lies in the range of AA present in common beverages. The sensor was designed with the thin film as the transducer, the copper hexacyanoferrate as the sensing element with the sensing scheme described in the below reaction.

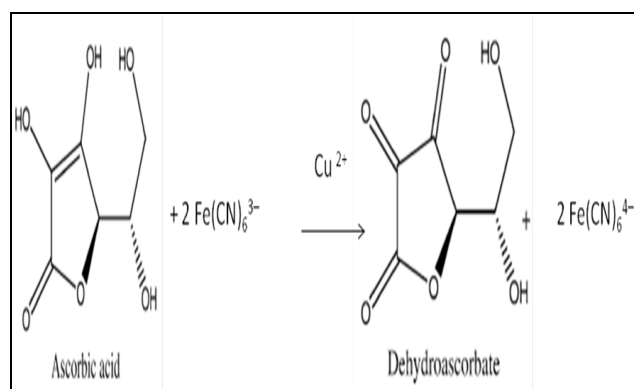


FIG. 1 REACTION MECHANISM OF ASCORBIC ACID AND COPPER HEXACYANOFERRATE

Experimental

Materials

Potassium ferricyanide (10 mM for spectrophotometric study and 0.6M for spin coating) and copper sulphate pentahydrate (10 mM for spectrophotometric study and 0.6M for spin coating) purchased from Keerthi Chemicals Ltd. and used as precursor salts for copper

hexacyanoferrate solution with Millipore water. Acetone & millipore water were used as solvents for ultrasonication. Pure silver ingots/foils (99.999%) were used as targets for thermal evaporation on Glass 2 x 1.5 cm² substrates. Different test solutions of AA of different concentrations from a stock solution of 1 mg / ml with millipore water as solvent.

Instruments

PerkinElmer Lambda 25 UV spectrophotometer was used for spectrophotometric studies. Ultrasonicator, PCI Analytics and Thermal Evaporation coating unit, AVAC engineers were used for cleaning substrates using ultrasonication and thermally evaporating silver ingots onto cleaned glass substrates, respectively. KC-605 LCR meter, Kokuyo Electronics Co Ltd. and Electrometer were used for resistance measurements. Surface Profilometer, Mutoyoto Electronics Ltd. was used for measuring thickness of coated films.

Procedure

1) Resistor Fabrication

The glass substrates were cleaned in running tap water, soap solution and ultrasonically cleaned with Millipore water for a minute, followed by ultrasonically cleaning them for a minute with acetone as the solvent in the ultrasonicator. The slides were dried after cleaning with acetone dipped cotton. The Silver sheet was cut into small strips. Silver strips were then cleaned by washing in tap water and dipping in acetone and then dried. The cleaned substrate and the Silver metal targets were placed inside the Vacuum chamber and a vacuum level of 1.9×10^{-5} mbar was created using rotary pump / diffusion pump. High voltage was supplied to create a primary current of 0.46 A and secondary current of 37.4 A for 4 minutes, during which the Silver target melts and then evaporates to get deposited on the glass substrate placed above in the substrate holder. The substrates were taken out after a cooling time of 15 mins in the vacuum chamber. The Silver coated slides were stored overnight. The optimized concentration of Copper Hexacyanoferrate (III) (CuHCF III) was found experimentally to be 0.6 M after trying different concentrations of CuHCF III (0.5 – 1M) for spin coating at 4000 rpm. 0.6 M CuHCF III was prepared by dissolving 1.8 g of Potassium Ferricyanide (0.6 M) and 1.2 g of Copper Sulphate pentahydrate (0.6 M) in 30 mL of double distilled Millipore water. The Silver coated glass slides are cut into 2.0 cm x 1.5 cm by a diamond cutter. 0.4

mL of 0.6 M CuHCF III was dispersed equally onto the Silver coated slide by spin coating. The spin coated slides were air dried for 15 mins and then stored in vacuum dessicator overnight. The thin film resistor fabrication was over with the leads kept by Silver paste. The distance between the leads was 1.5 cm. The resistor was stored in vacuum dessicator. The resistor was checked for its base resistance and open circuit voltage after connecting the leads to the LCR meter. Different concentrations of AA were prepared. 0.1 mL of each concentration was taken as sample volume and resistance change was observed. The resistance range, the minimum value and maximum value of the resistance value in the electrometer for every concentration was noted.

2) Spectrophotometric Study

10 mM solution of CuHCF III was prepared by dissolving 12 mg of Copper Sulphate pentahydrate and 16 mg of Potassium Ferricyanide in 10 mL of double distilled Millipore water. Different concentrations of AA (0.0625, 0.125, 0.250, 0.5 and 1 mg / mL) were prepared by appropriate dilution of the stock solution (1 mg / mL) with double distilled Millipore water. 3 mL of CuHCF III solution is added to Ascorbic Acid solutions. After 10 mins, the solutions were scanned at a speed of 120 nm / s in UV spectrophotometer with water as blank. The $\lambda_{\max}$ value was identified from the spectrum and the absorbance at that specific wavelength with respect to different concentrations was noted.

3) Resistor Sensing Scheme

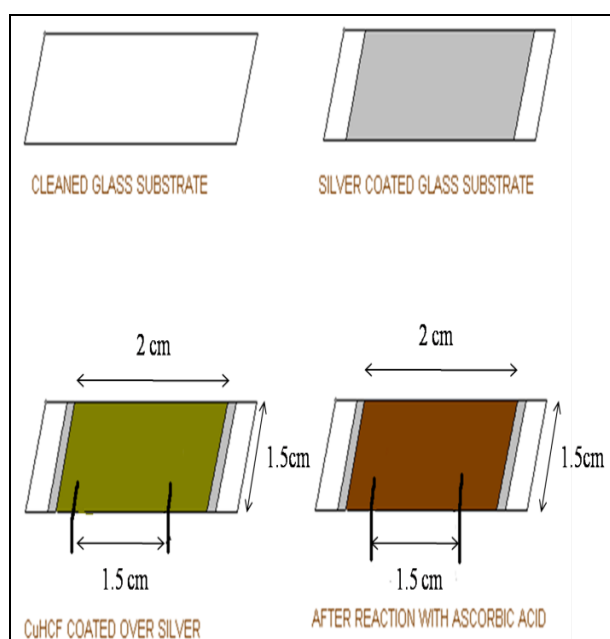


FIG. 2 (1a) CLEANED GLASS SUBSTRATE. FIG. 2 (1b) SILVER

THIN FILM COATED OVER THE GLASS SLIDE BY THERMAL EVAPORATION. FIG. 2 (1c) SILVER THIN FILM SENSOR WITH COPPER HEXACYANOFERRATE (III) SPIN COATED OVER THE THIN FILM AND THE LEADS WERE KEPT BY SILVER PASTE. FIG. 2 (1d) CHEMICAL SENSOR SHOWING GREENISH YELLOW TO DEEP BROWNISH RED COLOR CHANGE AFTER USE

Results

Sensor Priliminary Study

Silver thin film resistor was fabricated by evaporating pure silver ingots onto a cleaned glass substrate. The sensing element is then spin coated at 4000 rpm. The copper lead wires were kept by silver paste with a distance of 1.5 cm between them.



FIG. 3 FABRICATED RESISTOR

The feasibility study for the thin film resistor based sensor was performed with the initially fabricated silver thin film resistor coated with non-uniform layered CuHCF III with AC frequency of 100 Hz without any bias in the KC-605 LCR meter, Kokuyo Electronics Co Ltd.

TABLE 1 BASE RESISTANCE OF THE SILVER FILM RESISTOR WITH COPPER HEXACYANOFERRATE IS 49-52.7 MΩ WITH NO EXTERNAL BIAS, NON-UNIFORM COATING OF COPPER HEXACYANOFERRATE (III) AND WITH AC FREQUENCY 100 HZ. THE RESISTANCE RANGE WAS NOTED FOR CONCENTRATIONS OF 0.2, 0.4, 0.6, 0.8 AND 1.0 mg / mL OF ASCORBIC ACID AND TABULATED

SI no.	Concentration of Ascorbic Acid mg / mL	Resistance MΩ
1	0	50-54
2	0.2	49-52
3	0.4	44-48
4	0.6	39-43
5	0.8	38-44.2
6	1.0	39-45.5

The mean value of the resistance ranges was calculated for all the concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg / mL) of AA and plotted as Resistance v/s increasing concentrations of AA graph

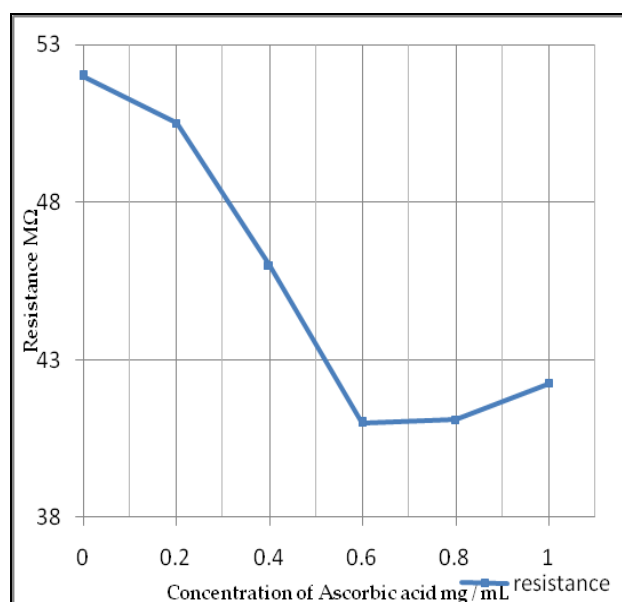


FIG. 4 THE RESISTANCE VS. CONCENTRATION OF ASCORBIC ACID GRAPH SHOWS DECREASE IN RESISTANCE WITH RESPECT TO CONCENTRATIONS OF ASCORBIC ACID FROM 0-0.6 mg / mL AFTER WHICH THERE IS SATURATION OF THE SENSOR WITH EXCESS ASCORBIC ACID. $R^2 = 0.815$

This experiment was done mainly to check the working of the resistor. A decrease in the resistance was observed with the increase in concentrations of AA without any change in the order of the resistance in MΩ. The decrease in resistance is observed when there is increase in concentration of AA confirmed the feasibility of thin film resistor based sensor.

Sensor Optimisation and Working

Concentration of CuHCF III was optimized to 0.6 M for Spin Coating. Uniform coating of CuHCF III over the Silver film was obtained. The surface profilometer was used to check the thickness of the silver thin film coated with copper hexacyanoferrate

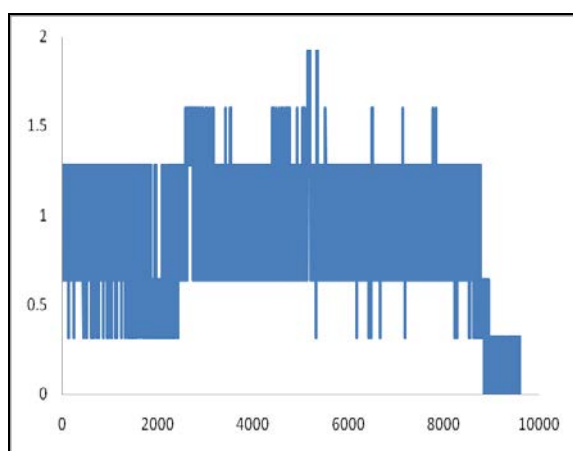


FIG. 5 THE SURFACE PROFILOMETER READINGS WERE TAKEN AND THE THICKNESS OF THE FILM WAS FOUND TO BE 1.3 MICRONS

Different concentrations of AA (0.02, 0.04, 0.06, 0.08,

0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 mg / mL) were prepared. 0.2 mL of each solution was the sample volume and resistance change for each concentration was observed.

The spin coated silver thin film resistor is analyzed for the change in resistance with increasing concentrations of AA. The decrease in the resistance of the sensor with respect to the addition of increasing concentrations of AA was even more effective in uniformly coated optimized resistor. The decrease in the resistance was from Giga ohms to Mega ohms (GΩ-MΩ) with respect to the increasing concentrations of Ascorbic acid at the bias of 50 mV. A cessation of change of resistance was experienced after 0.5 mg / mL.

TABLE 2 BIAS OF 50 mV DC VOLTAGE WAS GIVEN. THE OPEN CIRCUIT VOLTAGE WAS FOUND TO BE 0.26 - 0.3 V, BASE RESISTANCE IS 49.7-59 MΩ WITHOUT BIAS & 2.0 - 2.83 GΩ WITH A BIAS OF 50 mV. THE RESISTANCE RANGE, THE MINIMUM VALUE AND MAXIMUM VALUE OF THE RESISTANCE VALUE IN THE ELECTROMETER WAS NOTED

SI no.	Concentration of Ascorbic Acid mg / mL	Resistance Range MΩ
1	0.00	$2.7-3.35 \times 10^3$
2	0.02	$2.4-3.04 \times 10^3$
3	0.04	$1.4-2.0 \times 10^3$
4	0.06	$1.1-2.1 \times 10^3$
5	0.08	$0.25-0.442 \times 10^3$
6	0.10	$0.12-0.17 \times 10^3$
7	0.20	11.0-32.0
8	0.30	6.5-15
9	0.40	1.4-3.5
10	0.50	1.0-2.2
11	1.00	1.5-3.1

The mean value of the resistance ranges was calculated for all the concentrations of Ascorbic acid and plotted as Resistance v/s concentration of Ascorbic acid graph

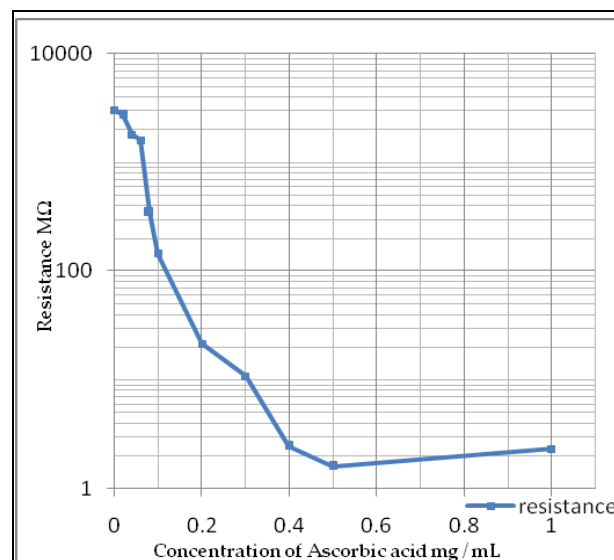


FIG. 6 THE GRAPH SHOWS DECREASE IN THE RESISTANCE

FROM THE ORDER OF GIGA TO MEGA OHMS WITH RESPECT TO DIFFERENT CONCENTRATIONS, 0- 0.5 mg / mL OF ASCORBIC ACID AFTER WHICH THE RESISTOR GETS SATURATED WITH EXCESS ASCORBIC ACID WITH $R^2 = 0.350$

Spectrophotometric Results

Different concentrations of AA (0.0625, 0.125, 0.250, 0.5 and 1 mg / mL) with 3 mL of CuHCF III were scanned at a speed of 120 nm/s in PerkinElmer Lambda 25 UV spectrophotometer with water as blank.

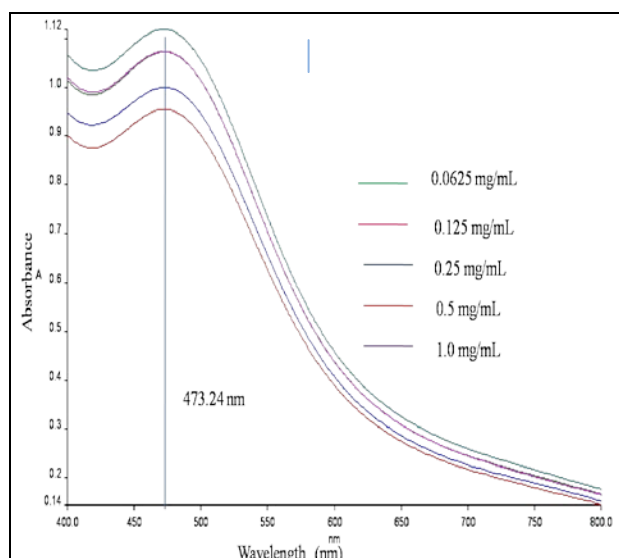


FIG. 7 THE ABSORPTION SPECTRUM OBTAINED FOR DIFFERENT SOLUTION IS SHOWN IN GRAPH. THE ABSORPTION SPECTRUM CLEARLY INDICATES THE ABSORBANCE MAXIMUM $\lambda_{\max}$ is at 473.24 nm AND DECREASE IN THE ABSORBANCE AS THERE IS AN INCREASE IN CONCENTRATIONS OF ASCORBIC ACID

From the absorption spectrum obtained the maximum absorbance $\lambda_{\max}$ peak is seen in the visible region at 473.34 nm which is mainly due to the ferricyanide ion, which is a blue colored species in the CuHCF III complex used.

The absorbance is measured with respect to different concentrations of AA at 473 nm.

Instrument: PerkinElmer Lambda 25

Serial No: 101N8060906

Method: conc1

Ordinate mode: Single wavelength

TABLE 3 THE CONCENTRATION V/S ABSORBANCE GRAPH SHOWS DECREASE IN THE ABSORBANCE OF COPPER HEXACYANOFERRATE WITH ASCORBIC ACID SOLUTION OF CONCENTRATIONS 0-0.5 mg / mL, AFTER WHICH THERE IS AN INCREASE IN THE ABSORBANCE.

Sample ID	Concentration (mg/mL)	Ord. value
Conc1.A01	0.0625	1.3903
Conc2.A02	0.1250	1.3326
Conc3.A03	0.2500	1.2440
Conc4.A04	0.5000	1.1408
Conc5.A05	1.0000	1.0000

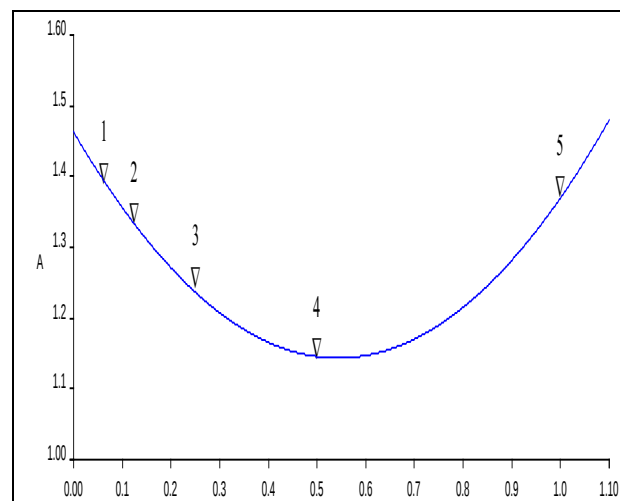


FIG 8 ABSORBANCE OF CuHCF III WITH RESPECT TO DIFFERENT CONC. OF AA AT 473 nm

The concentration v/s absorbance graph shows decrease in the absorbance of CuHCF III with AA solution of concentrations 0-0.5 mg / mL, after which there is an increase in the absorbance.

Discussion

Many graphite and nanoparticles modified electrodes have dominated the research for fabricating biosensor for detection of any substrate. Modified carbon nanotube electrodes have been used to sense Ascorbic acid with high precision in Worrapong Kit-Anan et al., 2012, Pablo R. Dalmasso et al., 2013 and Dilek Kul et al., 2013. Modified nanoparticles are also used for ascorbic acid sensing in Wentao Shi et al., 2012 and Geng-huang Wu et al., 2012. A simple, accurate, cheap and rapid sensing of AA was achieved in this technique. Silver can be easily obtained that would eventually bring down the manufacturing cost of the final quality control sensor. This technique opens up a new avenue to try other possible fabrication techniques.

In the resistor results, a color change from greenish yellow to deep brownish red is observed. The color change and the resistance decrease is mainly due to the formation of ferrocyanide ion from the redox reaction due to gain of two electrons by CuHCF III from AA. The sensitivity is found to be maximum during external positive bias of 50 mV. Hence the operating voltage of the sensor for effective sensing was 0.05V. It is mainly due to better pulling of electrons from AA to the CuHCF III film. The linear working range of sensor is 0-0.5 mg / mL. The sensor can be reused for 7-8 samples. The resistor has to be stored in vacuum dessicator to prevent reduction of CuHCF III on continuous exposure to air. The resistor

should be used within 4-6 days of its fabrication. It was mainly due to tarnishing of Silver which reduces the sensing element.

In the spectrophotometric results, the decrease in the absorbance is mainly due to the using up of ferricyanide due to its reduction to ferrocyanide facilitated by electrons from Ascorbic acid, which acts as a reducing agent. Cu^{2+} from the CuHCF III plays an important role in the oxidation of Ascorbic acid as a catalyst. Hence the entire spectrophotometric analysis is based on the catalytic oxidation of AA to dehydroascorbate with the reduction of ferricyanide to ferrocyanide. The increase in absorbance after 0.5 mg / mL can be reasoned as presence of unreacted ferricyanide ions as a result of saturated concentration of AA. Thus, the results correlates the linear working range of the sensor was spectrophotometrically found to be 0 - 0.5 mg / mL. The results from the resistance measurements and spectrophotometry are comparable in the range of 0-0.5 mg / mL where CuHCF III converts AA into dehydroascorbate. At higher concentrations of AA, there is saturation at the electrode in the resistance measurements and shown by increase in absorbance due to accumulation of ferricyanide which was evident in spectrophotometric analysis.

The results prove the capability of silver thin film resistor as an effective detection tool for sensing the levels of AA. The initial feasibility study clearly showed that the ability of silver thin film to adhere to glass substrate and serve as a transducing element. It was also evident that the CuHCF III was able to form a coated layer over the silver thin film and the electron transfer is effectively communicated to the electrodes. The non-uniform coating of CuHCF III in the feasibility was able to provide decrease in resistance with respect to the increasing concentration of ascorbic acid. In optimization of the sensor, spin coating was employed for the uniform coating of CuHCF III. The profilometry analysis showed a consistent coating of CuHCF III over the silver thin film. The optimized sensor model with improved coating of CuHCF III over silver thin film was able to provide change in resistance from Giga ohms to Mega ohms. The sample volume required for the sensing is 0.2mL and the bias voltage, 50mV. The color of the CuHCF III sensing element changed from greenish yellow to brownish red color after being used for 10-15 samples. The change in color indicated the complete reduction of CuHCF III from ferricyanide to ferrocyanide after being used for 10-15 samples. The effective re-usability

range of the sensor was determined to be 7-8 samples. It was evident from the color change that the electron transfer was mainly due to the electro-chemical oxidation-reduction reaction. The spectrophotometric results of decrease in absorbance at 473 nm with respect to the increasing concentration of ascorbic acid also emphasized that the oxidation reduction reaction was mainly due to the interaction between ascorbic acid and CuHCF III. However further investigation is required to articulate the effectiveness of the sensor such as analyzing real time samples, cross reactivity test. Nobler metals than silver can even provide a better transduction and increased longevity of the sensor.

Conclusions

A silver thin film resistor based sensor was developed for quantification of ascorbic acid for quality control of commercial fruits and fruit juices. The operational range of the sensor was found to be 0-0.5 mg/mL. The operating voltage of the sensor for effective sensing is 0.05 V. The reusability of the sensor is restricted to 8 samples. The resistor should be used within 5 - 6 days of its fabrication. The reusability and the usage restriction is due to tarnishing of silver. Future work is to improvise the sensor by using Platinum or Gold coatings. The specificity can also be improved by using ascorbate oxidase enzyme as the sensing element in the place of copper hexacyanoferrate.

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